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Effects of Four Combinations of Temperature and Daylength on the Ovogenetic Cycle of a Low-latitude Fish, *Fundulus confluentus* Goode & Bean[1]

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(Text-figures 1-4)

I. INTRODUCTION

EXPERIMENTS on the interplay of ecologic and endocrine factors in fish reproduction indicate that environmental influences on the sex cycle are reflected with greater dependability and particularity in the ovary than in the testis. In fishes as far as known, there is consistent correspondence between phases of the ovogenetic cycle and of the annual reproductive cycle, so that, normally, onset of spawning shortly follows completion of maturation by the vanguard of maturing eggs. Although similar timing holds for the spermatogenetic cycle in some fishes (Harrington, 1957; *et alii*), in others spermiogenesis is completed in autumn with spermiation postponed to spring (Turner, 1919; Kulaev, 1927, 1944; Harrington, 1956), and in still others there is year-round spermatogenesis with no evident change in tempo despite a marked annual ovarian cycle (Ghosh & Kar, 1952). The last condition is approached by *Fundulus confluentus,* and may prove common among low-latitude fishes. Subtler testicular changes, more precisely correlated with the annual emergence of nuptial behavior and color and onset of spawning, supposedly occur, but so far have not been satisfactorily demonstrated. The interstitial Leydig cells, presumed to elaborate androgen, and now deemed widespread among fishes (Marshall & Lofts, 1956), have proved difficult not only to interpret but to recognize in many species. Attention here will be devoted to the ovarian cycle.

Previous experiments on the relation of the environment to the sex cycle of fishes, with one partial exception (*vide infra* Hubbs & Strawn, 1957), were conducted on fishes local to latitudes 41° to 60° North (see complementary reviews of Atz, 1957; Harrington, 1959b). The present experiment was carried out at 27° North Latitude (Vero Beach, Florida), on locally indigenous specimens of the Marsh Killifish, *Fundulus confluentus* Goode & Bean. This species inhabits brackish waters along the whole Florida coastline and northward coastwise to Chesapeake Bay, occasionally entering fresh waters (Miller, 1955), where it is capable of reproducing (Harrington & Haeger, 1958; Harrington, 1959a). It has been reared in freshwater aquaria from egg to maturity and on into a second generation by the writer, but since it frequents salt marshes and mangrove swamps often far removed from fresh waters, oviposition must occur commonly in brackish water, although direct observations are lacking. Its well-known congener, *Fundulus heteroclitus,* ranges southward from Newfoundland, but is subject to the same climatic conditions in the southernmost part of its range. The two species are sympatric from Chesapeake Bay to the Matanzas River, in northeastern Florida, or throughout the range of *F. confluentus,* if the closely similar *F. grandis* be judged a subspecies of *F. heteroclitus,* which remains to be decided (Brown, 1954, 1957). Experiments concerning effects of extrinsic factors on reproduction in *Fundulus* have been confined to northern representatives of *F. heteroclitus* (Matthews, 1939; Burger, 1939, 1940), and have dealt exclusively with the spermatogenetic cycle. Since these were without specific reference to nuptial behavior and color or to sper-

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miation, and since the relation of the environment to the ovarian cycle was not studied, intrageneric comparisons with reference to latitude will not be possible.

The most appropriate season for testing effects of environmental factors on successive phases of the annual reproductive cycle is the non-breeding season, at the outset of which the ovaries—and in many fishes the testes also—undergo their maximum annual regression. This season is strikingly shorter at low than at high latitudes for fishes as for many other vertebrates. In Florida, *F. confluentus* evidently spawns soon after the end of January and continues into October, a breeding season much the same as that of the Pigmy Seahorse, *Hippocampus zosterae,* so instructively worked out in Florida by Strawn (1958). In contrast, the breeding season of *F. heteroclitus* in New England is June to early August (Bigelow & Schroeder, 1953). This leaves annually a non-breeding season of almost nine months for *F. heteroclitus* in New England but one of scarcely three months for *F. confluentus* in Florida, and brings to the fore the problem of the so-called refractory or postspawning period of fishes (*cf.* Harrington, 1957, 1959b).

The brief annual hiatus between successive breeding seasons at low latitudes affords little time for experimental maneuver. The duration of environmental conditions typical of the non-breeding season will be further curtailed by imposing within it variant experimental conditions, some of which might be expected to induce unseasonable changes in the course of maturation within the range of oocyte phases. Quantitative measurement of egg maturation, found useful in experiments at high latitudes (Bullough, 1939; Harrington, 1956, 1957), is all the more needed for effective analysis of results from experiments at low latitudes, because of possibly greater asynchrony in ovarian maturation among fish exposed to experimental conditions within the shorter annual interval between normal breeding seasons.

Before describing results of the present experiment, it will be necessary to define a sequence of oocyte phases as reference points for measuring quantitatively the stage-by-stage progress of ovogenesis within each group of fish exposed to a particular temperature-daylength combination.

II. Material and Methods

The experiment was begun December 15 and ended 45 days later, on January 31. Unforeseen difficulties prevented starting it December 1, as planned, but whether this would have sharpened the contrast in maturity at the end of the experiment between the most advanced experimental fish and those in the wild can only be conjectured. It appears most likely that had the experimental period been further postponed after the preceding spawning season, it would have ended so late as to greatly reduce the potential maximum contrast between a possible precocious maturation under any of the variant experimental conditions and the status of maturation in the wild.

Marsh Killifish were allocated to the four 20-gallon experimental aquaria on December 15, after having been acclimated for three days by gradually replacing the brackish water in which they had been collected with fresh. Since this species was found to spawn in fresh water both in aquaria and in the wild, the fish were changed to fresh water to avoid the further elaboration of apparatus required to minimize the tendency of brackish water in aquaria to become foul. Others of these acclimated fish were sacrificed the same day (December 15 controls). On January 31, fish fresh from the wild (January 31 controls) were sacrificed together with all fish in the experimental aquaria.

Two aquaria were kept in one bioclimatic room and two in another. Both rooms were sealed from daylight and regulated to maintain a water temperature of 15 ± 1° C. In each room, one aquarium was left at 15 ± 1° C. and the other kept at 30 ± 1° C. by means of a thermostatically-controlled aquarium heater. All illumination was from two fluorescent lamps (Westinghouse 40-watt Daylight), suspended 30 inches above each aquarium bottom; the light intensity at the bottom of the aquarium when empty was 753 lux. One room was illuminated for seven and the other for 15 hours each day. Each aquarium was supplied with an aerator and a charcoal filter; the fresh water filling it was not changed during the experiment, but well water was added to compensate for evaporation. Plants tolerating dim light (*Cryptocoryne* spp.) were rooted in the bottom sand.

This cover failed to allay the initial excitement of the wild fish, and a few bruised their snouts against the glass. When the killifish became habituated to captivity, all injured ones were removed. There was no mortality or sickness after the removal of the fish injured at the outset of the experiment. The numbers of female killifish in each of the four aquaria at the end of the experiment were 15, 17, 15 and 14, respectively (Table 2). In addition, each aquarium contained eight to ten males.

The fish were fed *ad libitum* once daily on

live mosquito larvae (*Aëdes* spp.), alternated with live daphnia and supplemented with mosquito larvae frozen in small blocks. The latter were ingested gradually as they fell from the melting blocks. Live food in excess of immediate demands remained in the aquaria to be consumed later, affording ample food for all fish by mitigating the influence of social dominance on feeding.

The standard lengths of all fish connected with the experiment were measured with vernier calipers to the nearest tenth of a millimeter. Originally selected for adult size, all proved much larger than the smallest that have spawned viable young in our aquaria. Each fish was weighed on a beam balance to the nearest hundredth of a gram, after removal of surface water with filter paper. Upon dissection and after remaining briefly on filter paper, the gonads were rapidly weighed on a Roller-Smith balance to the nearest two-tenths of a milligram, then fixed in Bouin's solution. After dehydration in an alcohol series, clearing in xylol, and transfer to cedar oil, the ovaries while in oil were teased apart, and the largest 50 egg diameters measured with an ocular micrometer. The testes were sectioned and stained with Heidenhain's iron haematoxylin, but as anticipated above, showed no obvious weight, size or histological differences, seasonal or otherwise, and will be left out of further account. If there is a seasonal change in the testes of *F. confluentus,* it is even more transitory than in those of *Oryzias* (Egami, 1956) and of little diagnostic utility in studies like the present one. In this respect *F. confluentus* differs from *F. heteroclitus,* the northern representatives of which, at least, have an obvious seasonal testicular cycle (Matthews, 1938; *et alii*).

Nuclear diameters as well as egg diameters were measured on a series of eggs from a representative fish of each experimental group and of both December 15 and January 31 controls. The nucleus is easily measured in cleared eggs of all phases of maturation up to the stage of yolk consolidation, for which there are no data on nuclear diameters. Since the distribution of nuclear diameters at each egg diameter was similar for corresponding egg diameters of different ovaries, the data for the fish of all groups were pooled (Table 1 and Text-fig. 1). Both egg and nuclear diameters were measured along whatever axis fell at random along the micrometer scale, a procedure sanctioned by the statistical analysis of Clark (1925). Sections of additional ovaries, fixed and stained the same as the testes, were examined to determine the approximate egg-diameter ranges corresponding with successive morphological phases of the ovogenetic progression.

The nucleoplasmic index at each egg diameter was computed from the mean nuclear diameter at each egg diameter, by the conventional formula, in which nuclear volume is divided by the difference between egg volume and nuclear volume. Egg and nucleus were treated as spheres, their volumes being computed from their diameters by the formula, $\pi D^3/6$, or $0.524\ D^3$. The gonosomatic index of each fish was obtained by multiplying the weight of the pair of gonads by 100, and dividing by the body weight.

III. Oocyte Phases

A. Defined by Egg Diameters Related to Changing Nuclear Diameters and to Yolk Consolidation

The changes in nuclear diameter with increasing egg size are recorded in Text-fig. 1. Each unit on the egg-diameter scale, along the x-axis, and of the nuclear-diameter scale, along the y-axis, equals 23 microns. In Text-fig. 1a, the distribution of empirical nuclear diameters at each egg diameter is plotted as a vertical row of dots. Each dot, according to its size, represents from one to 11 nuclei of the same diameter. In Text-fig. 1b, each hollow dot, determining the heavy line, records the mean nuclear diameter at a particular egg diameter. Each solid dot, determining the light line, records the nucleoplasmic index computed from a particular egg diameter and its corresponding mean nuclear diameter. The scale of nucleoplasmic indices is to the right. The data plotted in Text-fig. 1b are correlated with additional data in Table 1.

With graded increase in egg size, the nuclear diameter first undergoes a rapid increase, followed by a gradual decrease, and then tends to level off. Eggs of diameters greater than shown in Text-fig. 1 are in an advanced phase of maturation and so obscured by yolk that in cleared eggs the nucleus cannot be measured. With a shift in the growth differential between egg and nuclear diameters, the nucleoplasmic index begins its sharp decline somewhat before the rapidly enlarging nucleus reaches maximum diameter; the decline continues while the nuclear diameter is subsequently on the decrease, and approaches its minimum asymptotically as the nuclear diameter levels off. Although increase in egg diameter is a function of time, the egg-diameter scale conforms only to time sequence, not to time intervals.

At the outset of the series of successively larger eggs through which the nucleus pro-

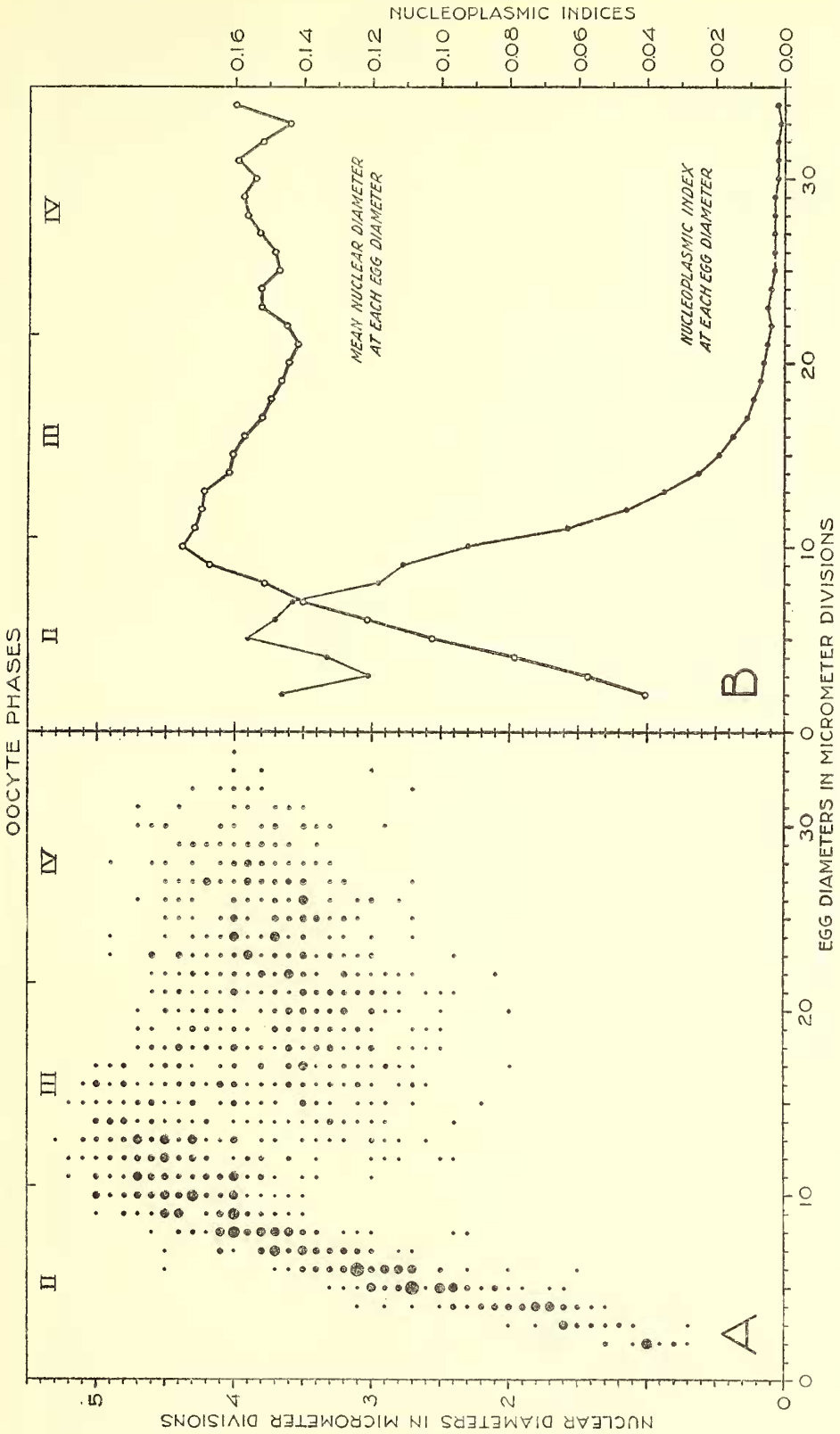
Table 1. The Correlation of Nuclear Diameter and Nucleoplasmic Index with Egg Diameter in Maturing Eggs of *Fundulus confluentus*. Compare Text-fig. 1.

Egg[1] Diameter	Number of Eggs Measured	Corresponding Nuclear Diameters[1]				Nucleoplasmic Index (Computed from Mean Nuclear Diameter)	Oocyte Phase
		Mean	Median	Max.	Min.		
2	18	1.01	1.00	1.3	0.7	0.146	
3	23	1.43	1.47	2.0	0.7	0.121	
4	52	1.96	1.91	3.1	1.3	0.133	
5	60	2.56	2.55	3.3	1.6	0.156	II
6	61	3.03	3.06	4.5	1.5	0.148	
7	52	3.50	3.49	4.5	2.7	0.143	
8	60	3.78	3.82	4.6	2.3	0.118	
9	48	4.18	4.11	5.0	3.5	0.111	
10	57	4.38	4.38	5.0	3.5	0.092	
11	55	4.29	4.30	5.2	3.0	0.063	
12	53	4.24	4.43*	5.2	2.4	0.046	
13	56	4.22	4.35*	5.3	2.6	0.035	
14	41	4.04	4.20*	5.0	2.4	0.025	
15	43	4.01	4.04	5.2	2.2	0.019	
16	58	3.93	4.00	5.1	2.6	0.015	III
17	49	3.80	3.64*	5.0	2.0	0.011	
18	56	3.74	3.67*	4.7	2.5	0.009	
19	44	3.66	3.68	4.7	2.5	0.007	
20	50	3.61	3.67	4.7	2.0	0.006	
21	50	3.54	3.49	4.6	2.4	0.005	
22	42	3.62	3.65	4.6	2.1	0.004	
23	47	3.81	3.86	4.9	2.4	0.005	
24	31	3.81	3.78	4.9	2.7	0.004	
25	35	3.68	3.63	4.5	2.7	0.003	
26	27	3.71	3.67	4.7	2.8	0.003	
27	38	3.82	3.85	4.5	2.7	0.003	
28	22	3.91	3.89	4.9	3.3	0.003	IV
29	19	3.94	3.90	4.4	3.4	0.003	
30	17	3.85	3.74	4.7	2.9	0.002	
31	9	3.98	3.95	4.7	3.5	0.002	
32	6	3.80	3.50	4.3	2.7	0.002	
33	3	3.60		4.0	3.0	0.001	
34	1	4.00		4.0	4.0	0.002	

[1] One unit = 23 μ. * See text.

gressively decreases in size (Text-fig. 1b), the empirical data show a pronounced increase in the range of nuclear diameters (Text-fig. 1a). Nuclear diameter distributions are at first skewed toward the smaller diameters, then a less evident countertendency sets in (*cf.* also the starred medians in Table 1), but there is no consistent skewness after the mean nuclear diameter reaches its minimum. The spread of nuclear diameter distributions is no doubt somewhat enhanced by the measurement at random along axes of ovoid nuclei and by the incidence of aspherical eggs, although the maturing ovaries of the Marsh Killifish are less compact and developing eggs depart less from spherical shape than in other fishes examined by the writer. The rather abruptly increased spread in nuclear diameter distributions is ascribable mainly to relatively short-time changes in nuclear volume. The present data do not rule out, but are insufficient to demonstrate, pulsations in the volumes of individual nuclei at a particular egg diameter, accompanying the gradual diminution of the mean nuclear diameter.

Hereafter, eggs with diameters ranging up to 10.9 units will be referred to oocyte phase II within which the nucleus increases in size; eggs with diameters between 11 and 21.0 units, to oocyte phase III within which the nucleus decreases in size; eggs with diameters from 22 to about 34 units, to oocyte phase IV within which nuclear diameters tend to level off; and all larger eggs, to oocyte phase V which includes mature eggs (compare Tables 2-3 and Text-figs. 2-4).



TEXT-FIG. 1. Interrelations of oocyte phase, egg diameter and nuclear diameter in *Fundulus confluentus*. One micrometer division = 23 μ. Compare Table 1. (A) Range of nuclear diameters at each egg diameter. Each dot, according to size, records from one to 11 measurements. (B) Mean nuclear diameter and nucleoplasmic index at each egg diameter.

B. Significance

The oocyte phases defined above approximate those given the same numerical designations by Detlaf & Ginzburg (1954), but established on a morphological basis (*cf.* Ivanov & Dodzina, 1957) by Meien (1939), who named them, respectively, the one-layered follicle phase, the phase of the primary accumulation of yolk, the phase of the filling up of the oocyte with yolk, and the phase of the ripe oocyte. In this classification, oocyte phase I is known as the juvenile phase and is characterized by, among other things, an at best incomplete follicular epithelium in which the nuclei of the epithelial cells investing the oocyte are remote from each other. Based on complexes of these oocyte phases, a series of ovarian maturation stages are defined by Russian workers, which will be referred to hereafter merely as ovarian stages. To simplify, the most advanced oocytes in *ovarian stages* I to V are in *oocyte phases* I to V, respectively. With allowance for differences among fish taxa and difficulties of precisely establishing the extreme diameter limits of each oocyte phase, our oocyte phases correspond with those similarly designated by Detlaf & Ginzburg (1954) as oocyte phases II to V. Trusov (1947) subdivides ovarian stage IV in the European Pike-perch *(Lucioperca)* into IV-A, IV-B, and IV-C (actually labelled by the first three letters of the Cyrillic alphabet), in effect limiting stage V to that condition of the ovary in which the ripest eggs have left their follicles. The transition from late oocyte phase IV to phase V in *Fundulus confluentus* is so slight and rapid that the exact boundary between late ovarian stage IV and stage V is of little import in the present experiment, and is mentioned only to underscore the fact that our oocyte phase V includes oocytes corresponding to those typifying both Trusov's ovarian stages IV-C and V. Ovarian stage VI is characterized by the presence of empty (atretic) follicles, and is thus first encountered after onset of spawning. Our oocyte phases are numbered to permit comparisons with the apposite ovarian stages cited in the extensive Russian literature on fish reproduction. The writer otherwise would have followed the classification of fish oocyte phases used by Yamamoto (1956a), which is based on more thorough cytomorphological and cytochemical studies. Moreover, the present investigation concerns responses throughout a succession of oocyte phases, while the Russian nomenclature emphasizes ovarian stages. Although each ovarian stage is based on a *complex* of oocyte phases, this nomenclature diverts attention from the earlier oocyte phases to the most advanced oocyte phase of that complex of oocyte phases characterizing a particular ovarian stage.

Oocyte phases I and II belong to the lesser growth period of the oocyte, all later phases to the greater growth period, or period of more rapid growth (Meien, 1939; and subsequent Russian authors). These two growth periods evidently correspond with the primary and secondary growth periods (*e.g.* of Bullough, 1939) or the growth periods 1 and 2 (of Marza *et al.*, 1937). The secondary growth period is described and illustrated for *Fundulus heteroclitus* by Marza *et al.* (1937) as period 2, of which phases A_1 and A_2 approximate our oocyte phase III, phases B_1 and B_2, our oocyte phase IV, and phase B_3, our oocyte phase V. According to Sakun (1957), the period of trophoplasmatic growth includes the phase of primary accumulation of yolk, in which vacuoles are formed in the cytoplasm (our oocyte phase III), and the phase of the accumulation of yolk in the form of lipid-containing granules (our oocyte phase IV). Konopacka (1935) and Yamamoto (1955a, b; 1956e, f, g; 1958) specify that vitellogenesis proper begins with the first appearance of vacuoles. Mas (1952) equates these with his "plagues claires" and with the "proteinaceous yolk vesicles" described in *F. heteroclitus* eggs at the outset of growth period 2 (Guthrie, 1928, 1929; Marza *et al.*, 1937). Kazanskii (1951) describes ovaries in stage III, in which the advanced eggs had fluid-filled vacuoles but as yet no lumps of yolk. Bullough (1939) notes that growth beyond the primary growth period is coincident both with loss of cytoplasmic basophilia and with the appearance of vacuoles, in connection with which "yolk droplets" finally arise. Chopra (1958) describes clear vacuoles giving rise to 'vacuolar yolk,' which he equates with the "yolk vesicles," the name used for these vacuoles by Yamamoto (1955a *et seq.*), who found them to be chiefly composed of polysaccharides and who believed them finally to give rise to the cortical alveoli, which at fertilization are extruded into the interspace between plasma membrane and egg membrane.

The consensus of these works is that the beginning of the greater (secondary) growth period of the oocyte coincides with the onset of vitellogenesis, for which the *ab initio* appearance of cytoplasmic vacuoles (yolk vesicles) is diagnostic.

In sectioned eggs of *F. confluentus,* these yolk vesicles are first seen at egg diameters of about 10 units (*cf.* Text-fig. 1b), the egg diameter above which the nucleus begins to diminish in size, suggesting that reduction in

nuclear diameter is related to onset of vitellogenesis as expressed in the changes occurring within oocyte phase III, the phase of primary accumulation of yolk (yolk vesicle phase). The antecedent onset of the decline in the nucleoplasmic index (Text-fig. 1b) reflects a change in the differential between egg and nuclear growth rates, which may prove equally significant in the chain of events correlated with onset of vitellogenesis. It is soon after this that the yolk vesicles appear and the cytoplasm loses its basophilia (*cf.* preceding paragraphs).

The phenomenon of nuclear diminution recorded in Text-fig. 1 seems otherwise to have escaped notice in fish eggs. It would be difficult to detect from measurements on sectioned ovaries. Subramaniam & Aiyar (1935) noted a "progressive reduction in the size of the *nucleoli* [italics ours] with increase in the size of the fat-globules" in *Acentrogobius neilli.* Singh & Boyle (1938) twice misquoted this as a reduction in the size of the *nuclei,* which they associated with expulsion into the cytoplasm of nucleolar substances, inferred from their own studies to initiate the formation of the vacuoles (yolk vesicles). The validity of such extrusions in fish oocytes has been denied (Nath *et al.,* 1944; Nath, 1957), but Yamamoto (1955a, b; 1956b; 1958) presents topographical and cytochemical evidence of their occurrence and participation in yolk vesicle formation. According to Bonhag (1958), on the other hand, the general theory that nucleolar emissions play a role in yolk production awaits adjudication by ultramodern techniques. More recently, however, Hsu & Lou (1959) have demonstrated by time-lapse cinematography the extrusion of nucleolar material in mouse melanoma cells, in time sequences showing discontinuous output (Nuclear Pump Action). Moreover, they found that fresh nutrient was essential to the process, which points to a requirement for ribonucleic acid material from the nucleus for the synthesis of new material in the cytoplasm (*cf.* comments of discussants, *loc. cit.*). Of further interest in this connection is the hypothesis of Pantelouris (1958), derived from the study of radioisotope-labelled newt oocytes, that a protein migration from cytoplasm to nucleus predominates before vitellogenesis and migration in the opposite direction occurs during vitellogenesis.

IV. Experimental Results

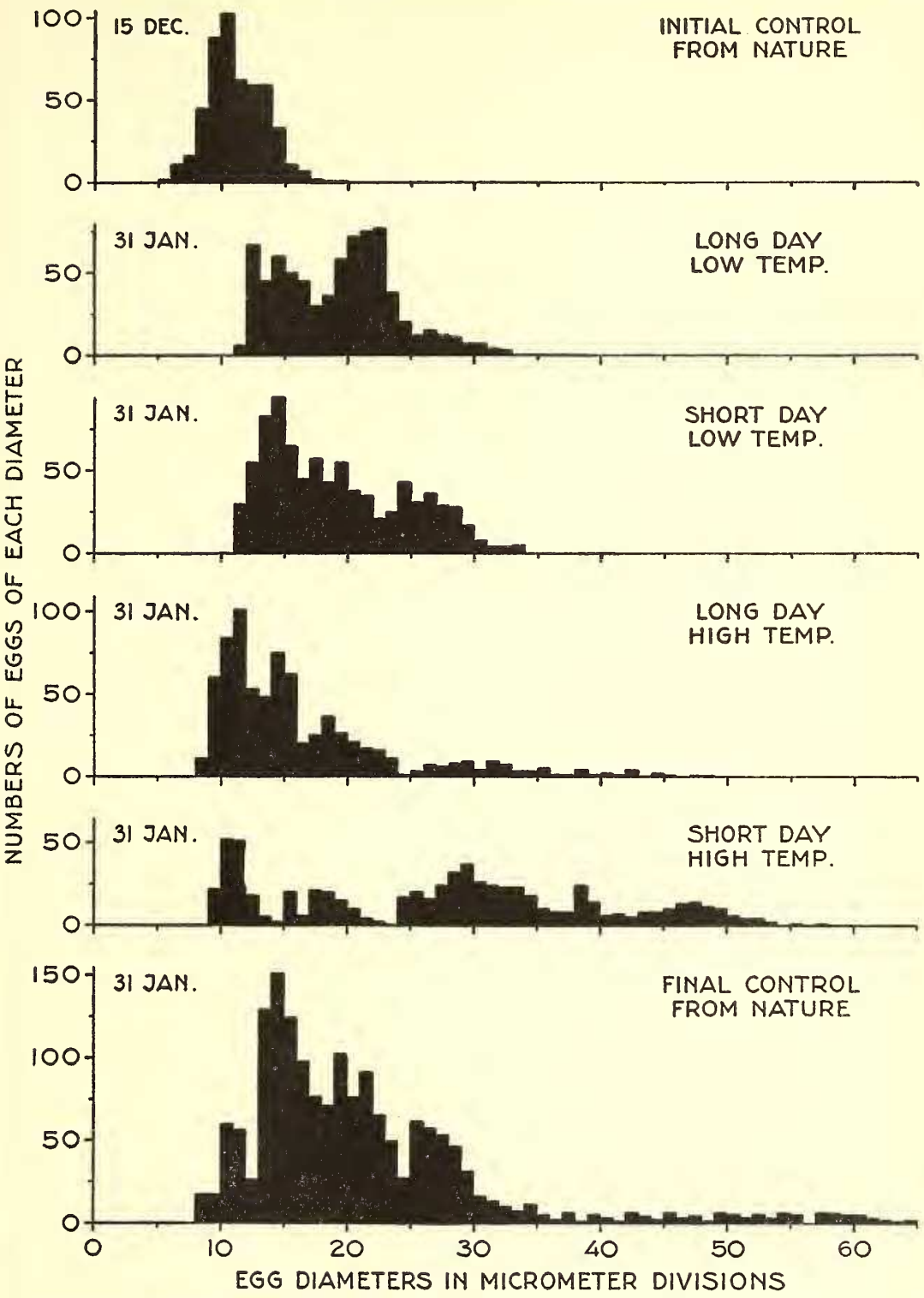
At the beginning of the experiment, the ovaries were in stages II and III, their largest oocytes being about equally divided between oocyte phases II and III (Table 2 and Text-fig. 4). The experiment was ended when the largest oocytes in the ovaries of the ripest fish were in our phase V (*vide supra* Oocyte Phases). Observation was intensified toward the end of the experiment to assure its termination before spawning began. Examination of the ovaries later confirmed that no fish had spawned. The experimental results can therefore be evaluated in terms of the progress of the largest 50 eggs per fish along the scale of egg diameters corresponding to oocyte phases II through V, paralleling the like-numbered ovarian stages, as well as with reference to gonosomatic index.

The frequency distributions of the largest 50 eggs per fish are pooled for each experimental group in Table 2 and illustrated in Text-fig. 2 for comparison with analogous histograms in the literature (*cf.* Bullough, 1939; Harrington, 1956, 1957, 1959b). The range of the largest 50 egg diameters is separately recorded for each fish as a vertical line in Text-fig. 3. The ranges within each experimental group are disposed in an ascending series with the more mature fish represented to the right and the more mature eggs toward the top. The scale of egg diameters is to the left. The hollow dot related to each vertical line records the gonosomatic index of the same fish, with the scale of gonosomatic indices to the right, in which the higher the value, the more mature the fish. Table 3 gives the frequency distributions of the largest 50 egg diameters separately for each fish of the two high-temperature groups, of which one was subject to a long and the other to a short daily photoperiod. Text-fig. 4 shows the percentages of all eggs measured for each experimental group (the group sum of the largest 50 per fish) found in oocyte phases II to V, respectively, as computed from the data in Table 2. To shorten circumlocution, the largest 50 egg diameters per fish will often be referred to as the vanguard eggs.

The general character of the group response to each of the four variant treatments, as well as the status of maturation in the wild at the beginning and end of the experiment, is apparent from Table 2 and Text-fig. 2. In contrast to experimental constant temperatures of 15° C. or 30° C. and fixed daylengths of 7 hours or 15 hours, the terminal (January 31) controls were subject in the wild to fluctuating temperatures closer to the low than to the high experimental temperature and to daylengths closer to the short than to the long experimental daylength. In December, the average air temperature locally was 19.1° C. (av. daily max., 23.3° C.; av. daily min., 13.1° C.); in January, it was 18.9° C. (av. daily max., 24.4° C.; av. daily min., 11.7° C.). During the 45-day experi-

TABLE 2. EFFECTS OF DAYLENGTH AND TEMPERATURE ON THE MATURITY OF *Fundulus confluentus*. The frequency distributions of the largest 50 egg diameters per fish pooled for each group. One egg-diameter unit = 23μ. Compare Text-fig. 2.

Egg Diameters	Control Dec. 15 (10 Fish)	15° C.		30° C.		Control Jan. 31 (33 Fish)	Oocyte Phases
		15-hr Day. (15 Fish)	7-hr. Day (17 Fish)	15-hr Day. (15 Fish)	7-hr. Day (14 Fish)		
5	2						
6	11						
7	16						
8	n= 45	n=	n=	n= 11	n=	n= 17	II
9	265 88	0	0	155 60	74 22	93 17	
10	103			84	52	59	
11	62	6	28	101	51	56	
12	59	67	55	53	18	26	
13	59	45	83	48	5	129	
14	33	60	95	75	2	151	
15	n= 11	n= 50	n= 65	n= 62	n= 20	n= 124	III
16	235 7	544 45	599 45	484 20	172 6	999 97	
17	2	30	57	25	21	76	
18	1	36	43	36	20	71	
19	1	58	55	26	15	102	
20		72	38	21	10	76	
21		75	35	17	4	91	
22		77	21	16	2	65	
23		38	25	11		49	
24		20	43	1	17	27	
25		12	31	3	20	61	
26		15	36	7	16	57	IV
27	n=	n= 12	n= 29	n= 6	n= 24	n= 53	
28	0	206 11	251 28	87 8	262 32	446 46	
29		7	17	9	37	31	
30		7	8	4	26	16	
31		4	4	9	24	13	
32		3	4	7	23	10	
33			5	3	23	7	
34				3	18	11	
35				5	10	5	
36				1	8	2	
37	n=	n=	n=	n= 1	n= 8	n= 6	V
38	0	0	0	24 4	192 24	112 1	
39				1	14	5	
40				2	6	3	
41				1	7	1	
42				4	5	6	
43					8	4	
44				2	8	2	
45				1	10	6	
46					13	3	
47				1	14	4	
48				1	11	1	
49					10	6	
50					6	5	
51					4	3	
52					4	5	
53					2	3	
54						6	
55					1	5	
56							
57					1	6	
58-64						24	



TEXT-FIG. 2. Histograms of effects of daylength and temperature on the ovaries of *Fundulus confluentus*. The diameters of the largest 50 eggs per fish pooled for each group are plotted from data in Table 2. One micrometer division = 23 μ.

TABLE 3. EFFECTS OF DAYLENGTH ON THE MATURITY OF *Fundulus confluentus* AT 30° C.

Egg[1] Diameters	Frequencies of the Largest 50 Egg-Diameters per Fish: 15-hour Day (15 Fish)															7-hour Day (14 Fish)													
8	11																												
	32	19	9													22													
10	6	19	22	27	10											25	27												
	1	9	15	13	26	23	14									2	16	33											
12		3	4	9	10	15	12									1	3	14											
				1	2	8	7	10	9	7	4						2	3											
14					2	2	7	17	18	17	12						2												
						2	4	16	17	10	13								20										
16							1	5	6	5	2	1							6										
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18							3			3	4	13	13						10	10									
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30															4						2	3	4	9	3	5			
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32														3	4						1	3	4	5	5		5		
															3							6	2	4	5	2	4		
34														1	2						1	2	1	4	1	5	4		
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[1] One unit = 23 μ.

mental period, natural daylengths declined from 10.5 to 10.4, then increased to 10.9 hours. The January control group resembles the two groups at high temperature in that the vanguard eggs comprise oocytes of phases II and V as well as intermediate ones. In only two of the 33 control fish have eggs reached phase V, however, and in only three are the van-

TEXT-FIG. 3. Effects of daylength and temperature on the ovaries of individual *Fundulus confluentus*. Each vertical line spans the largest 50 egg diameters of one fish; the related hollow dot records the gonosomatic index of the same fish. One micrometer division = 23 μ.

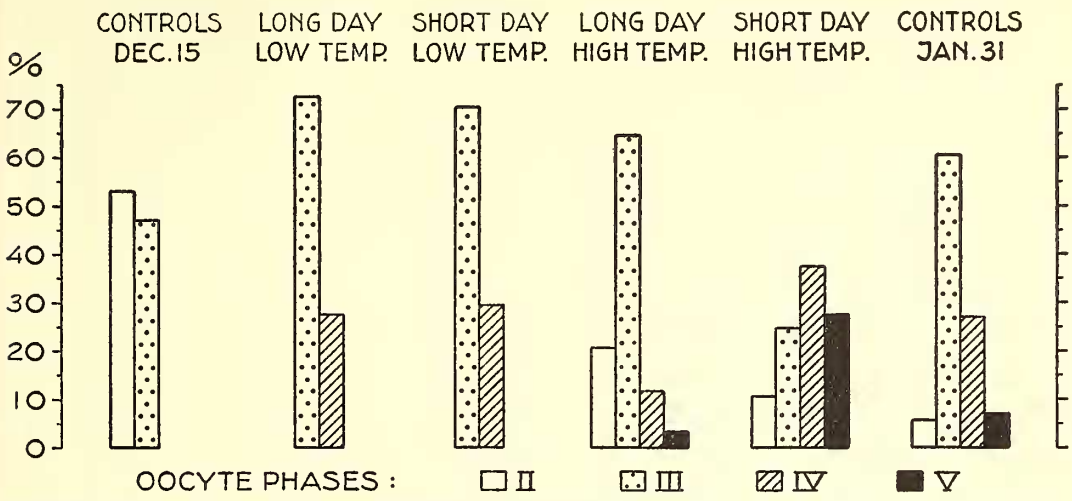
guard eggs represented in phase II (Text-fig. 3). The distribution of the vanguard eggs in the January 31 control group is otherwise much like that of both low-temperature groups, and may be regarded tentatively as intermediate between that of the short-day, low-temperature group and that of the long-day, high-temperature group.

The two low-temperature groups are closely similar, and differ in two respects from all other groups at the end of the experiment. The vanguard eggs in both have without exception passed entirely out of phase II into phase III or beyond, but none have reached phase V. In both high-temperature groups, on the contrary, vanguard eggs are represented by both phase II and phase V oocytes, as in the January 31 control group. The distribution of these maturing eggs, moreover, is suggestive of opposing tendencies, *viz.* a lag in development at smaller diameters and a speed-up at greater ones. Of the two groups at high temperature, the one subject to short days is by far the more advanced in maturity and, as will be demonstrated from several aspects, is by far the most advanced group in the experiment. At low temperature, on the other hand, there seems to be little if any difference in maturation with contrasting daylengths. The slight advance (within phase IV, compare Text-figs. 2 and 3) of short-day over long-day fish at low temperature derives its sole significance, if any, by analogy with the marked advance under short days at high temperature, since it is not statistically significant.

The above disposition of the experimental data reflects the extent and amplitude of each group reaction, and permits group-to-group comparison, only moderately blurred by the variation in numbers of fish per group (Table 2). It does, however, conceal the responses of individual fish. This omission is corrected by Text-fig. 3, in which the extent of the response of each fish is measured by the position and length of the vertical line spanning the range of its largest 50 egg diameters. The amplitude of its response is roughly expressed by its gonosomatic index, but despite general conformity between gonosomatic indices and later phases of egg maturation, the gonosomatic index proves to be a tardy indicator of the course of ovogenesis (Text-fig. 3), effectively registering progress in maturation only beyond oocyte phase III (*cf.* Text-fig. 1). Quantitative analysis of egg diameters is required to assess the amplitude and extent of maturation through oocyte phases I-III in this species, although in its much larger congener, *F. heteroclitus,* this may not prove necessary.

Text-fig. 3 also discloses the staggered pattern of responses by the fish within each group exposed to the same daylength-temperature combination. It further shows that in the low-temperature fish, the ovaries were either in stage III or IV, and that these fish can be arranged in an evenly-graded series with respect to maximum egg diameters attained in each, within the range of diameters encompassed by oocyte phases III to mid-IV. In contrast, the ovaries of both groups at high temperature (*cf.* Table 3) were either in stage III or V. Some of the stage-III ovaries contained vanguard eggs in oocyte phase II as well as in oocyte phase III, and their largest eggs were at most on the border line between phases III and IV, unlike the stage-III ovaries of the low-temperature fish. Most of the stage-V ovaries had vanguard eggs in oocyte phase IV in addition to those in oocyte phase V. The only high-temperature fish that can be evenly graded with reference to maximum egg diameters attained are those with no eggs in phase V. Beginning with phase IV, there is for the first time a sudden, pronounced extension of the distributional spread of the vanguard egg diameters (Tables 2-3 and Text-figs. 2-3). This sudden increase in the range of diameters comprehended by the largest 50 eggs no doubt reflects an abrupt change in the growth rate of larger eggs as they come under influences rapidly leading to definitive egg size (*cf.* figures of Harrington, 1956, 1957, 1959b; and Text-fig. 1 of Yamamoto, 1956a). In some fish (Text-fig. 3 and Table 3), these eggs range from diameters appropriate to the outset of phase IV to those equal or almost equal to mature eggs. This draws attention to the unprotracted ranges of the vanguard egg diameters in the low-temperature fish, including even those in which vanguard eggs have entered oocyte phase IV (Text-fig. 3).

When eggs that were measured in 70% alcohol after fixation in Bouin's solution are remeasured in cedar oil after clearing, the largest ones are found to have shrunk as much as 7-8 units on the present scale, or about 0.16-0.19 mm. Shrinkage is progressively less at smaller diameters, becoming negligible toward the smallest diameters of oocyte phase IV. This places the largest eggs in the experiment at the definitive size for the species. Living fertile eggs of *F. confluentus* measure about 1.6 mm. in outside diameter, and the largest eggs in the experiment, when corrected for shrinkage, measure $1.47+0.16=1.6$ mm. The egg diameters bounding each oocyte phase are only approximate, of course, but both dissected and sectioned ovaries were similarly fixed and cleared



TEXT-FIG. 4. Percentages of eggs measured for each experimental group of *Fundulus confluentus* found in oocyte phases II to V, respectively. For each group 100% represents the grand total of the largest 50 eggs per fish, *e. g.* 500 eggs for the December 15 control group of 10 fish.

so that their egg diameters could be compared. The small number of eggs approaching final maturity in the most mature fish of the experiment is consistent with the fact that we have been unable so far to strip and fecundate more than 25 eggs from a single female at one time.

Within each high-temperature group, a line of demarkation separates fish advanced in maturity from those retarded, with minimal overlap between the vanguard eggs of retarded fish, which are predominantly in phases II-III, and those of advanced fish, which are predominantly in phases IV-V. This effect is ascribable to high temperature, since no such separation occurs in the other groups, not even among the January 31 controls, although they exhibit the same range of oocyte phases. The two groups at high temperature are seen to differ from each other in amplitude and extent but not in character of response. The ratio of high-temperature fish with ovaries in stages III versus V is 13 : 2 in the long-day group and 5 : 9 in the short-day group (Table 3 and Text-fig. 3). The difference between these group responses (proportions) is significant. With Yate's correction, the value of Chi Square is such that $P<0.016$, and would doubtless be found somewhat less if computed by the laboriously exact method for 2×2 contingency tables. Table 3 shows in detail the amplitude of the response of each fish of these two groups, but the great advance in maturity of the short-day, high-temperature group over all others may be more clearly apprehended from Text-fig. 4.

In Text-fig. 4, the percentages of the largest 50 eggs per fish falling into each of the four oocyte phases (II-V) adds up to 100% for each experimental group. This permits group-to-group comparisons undistracted by the staggered responses of the individual fish and with a minimum of distortion from the moderate variation in numbers of fish per experimental group (*cf.* Table 2). It sums up the conformity of the two groups at low temperature, both showing no daylength influence, an accelerated transition from oocyte phase II to III-IV, and a lack of transition from phase IV to V. It registers the retardation at high temperature of the transition from oocyte phase II into III as well as the opposing accelerated progression toward definitive maturation in the larger eggs. The advance of the short-day, high-temperature group over all others is as unmistakable as it was unexpected. It clearly involves the concentration of vanguard eggs in oocyte phases IV and V, whereas in all other groups at the end of the experiment, including the final controls, the vanguard eggs are concentrated in oocyte phase III.

V. Discussion

Since the present experiment, with one partial exception (*vide infra* Hubbs & Strawn, 1957), is the only one of its kind conducted at so low a latitude, comparison of its results is virtually limited to those obtained with fishes from high latitudes. The high-latitude fishes studied spawn toward either end of that segment of the year occupied by the prolonged spawning season of low-latitude fishes, *viz.* one species in autumn, the rest in spring-summer. There is presumptive evidence that one spring

spawner, *Esox americanus vermiculatus,* bred again during an unseasonably warm autumn (Lagler & Hubbs, 1943), but fry from such adventitious spawning could not be expected to add effectively to the population. There is no reason, however, to minimize the biological importance of spawning by *F. confluentus* late in the season, for its eggs, even when stranded by receding waters toward the end of its spawning season, hatched when reflooded after having lain on sods out of water two to three months during an unusually cold winter (Harrington & Haeger, 1958; Harrington, 1959a). This projection of hatching far into the non-breeding season has been recorded only in other cyprinodont fishes in India, Africa and South America.

Baggerman (1957) found that four combinations of high or low temperature and long or short days induced in *Gasterosteus aculeatus* one pattern of positive and negative responses when applied to fish in an early phase of maturity and another pattern when applied to fish in a later phase of maturity. These phases are predicated on terminal behavioral criteria unaccompanied by gonad examination (*cf.* Harrington, 1959b), and have only general relevance here, but in so far as they furnish evidence of different responses by the reproductive mechanism to identical sets of extrinsic factors according to phase of maturity, they are of interest with respect to the different effects on earlier versus later oocyte phases found in the present study. Verhoeven & van Oordt (1955) discovered in *Rhodeus* a somewhat analogous difference in reaction to daylength and temperature according to phase of sexual maturity (cycle).

The inhibitory effect of high temperature on ovogenesis within earlier oocyte phases of *F. confluentus* finds a close parallel in the ovaries of *Apeltes* (Merriman & Schedl, 1941), *Phoxinus* (Bullough, 1939, graphs 10-11, not text; *cf.* Harrington, 1959b), and *Enneacanthus* (Harrington, 1956). In all experiments on other species, except *Notropis bifrenatus,* no attention was paid to the successive phases of ovogenesis, so that this reaction would have been overlooked. In the experiments on *N. bifrenatus,* however, all fish were kept at high temperature (Harrington, 1950, 1957). On short days, the eggs of *N. bifrenatus* increased in size up to a critical diameter, at which they stopped and beyond which they continued to final maturity outside the normal breeding season only with long days. Eggs reached the critical diameter sooner with long than with short days, and possibly at low temperature would have advanced even faster, but none began to exceed the critical diameter until mid-November. After this, long days at high temperature induced completion of the sex cycle (spawning) in six weeks, and when first imposed on other individuals January 1, they again induced spawning in six weeks. The effect of high temperature on the spawning of the low-latitude fish, *Etheostoma lepidum,* (Hubbs & Strawn, 1957), will be considered later in a more appropriate context.

The period before the end of which fishes respond neither to gonadotropin injection nor to environmental factors that otherwise induce spawning in the non-breeding season within a confined number of days has been called the refractory or postspawning period (*cf.* Atz, 1957; Harrington, 1959b). The refractory period seems to parallel that time interval during which the vanguard eggs are traversing some of the earlier oocyte phases. Present results, together with the ones cited above, suggest that in fishes this period can be shortened by subjecting them to that combination of external factors most favoring maturation through the early phases, after which the fish might be expected to respond at once to the different combination of external factors known to induce final maturation when imposed outside the natural spawning season. Both the critical egg diameter of *Notropis* and the disjunct distributions of the vanguard eggs of *Fundulus,* that were either retarded or accelerated in maturity by high temperature (Table 3 and Text-fig. 3), reflect a point below and above which there are different responses to a constantly maintained set of external conditions. This is intelligible, moreover, in the broader ecological context. It follows that both the term *refractory period* and any assertions that a given environmental variable is without influence on the sex cycle of a fish must be qualified as to the phases of maturation concerned.

If such a refractory period can be identified in *F. confluentus* at all, it can only be equated with the high-temperature retarded passage of oocytes from phase II (possibly also phase I) through III. Beyond phase III, the response to high temperature seems to change from negative to positive. Although eggs progressed to mid-phase IV at low temperature, at high temperature within the same time interval, those not kept from reaching phase IV continued through phase V toward final maturity (Tables 2-3 and Text-figs. 2-3). High temperature thus induced the later phases of maturity while retarding the earlier, and low temperature accelerated the earlier while arresting the later. There was no unmistakable influence by daylength at low temperature or on later phases of maturation at high temperature, but the retardation of

ovogenesis within the earlier oocyte phases by high temperature seems to have been strongly reinforced by long days (Table 3). It is worth mentioning here that in the European Pikeperch *(Lucioperca),* the transition from ovarian stage III to IV begins in October with the sudden fall in temperature (16.6° C. to 7.4° C.), lasts 1-1½ months, and is correlated with the resumption of rapid production and accumulation of gonadotropin (Trusov, 1947). The oocytes thereafter remain until January in the phase of primary accumulation of *fatty* yolk, which characterizes Trusov's ovarian stage IV-A, and was so named by him to distinguish it from the phase of primary accumulation of yolk as designated by other Russian authors, *i.e.* the phase of the formation of vacuoles or yolk vesicles (our phase III), which precedes it.

The refractory period of fishes is one of two alternate phases of an internal rhythm, even though its duration is conditioned by external factors. In *Notropis bifrenatus,* it begins long before temperature and daylength have declined to the levels at which they initiated breeding at the outset of the spawning season (Harrington, 1957). In *Gasterosteus aculeatus* under constant high temperature and long days there is an intrinsic 200-day reproductive rhythm, within which a reproductive period alternates with a non-reproductive (refractory) period (Baggerman, 1957). After the refractory period ends, breeding can be induced out of season by imposing a certain combination of external conditions (Harrington; Baggerman, *loc. cit.; et alii*), and the present results suggest that the refractory period can be shortened by imposing another different combination. Baggerman showed that in *Gasterosteus* there is no internal factor alone able to initiate a breeding period in the absence of an appropriate combination of external factors, but Bullough (1941) inferred an internal rhythm in *Phoxinus* capable of acting in the absence of the seasonal environmental conditions normally regulating the precise time of its action. When daylength was restricted starting early in the calendar year, *Phoxinus phoxinus,* which requires long days for maturation out of season, although retarded, still reached full breeding condition within its natural spawning season.

The over-all duration of spawning by *F. confluentus* populations at the same latitude is probably a matter of environmental regulation chiefly, although co-action of internal rhythm and external factors might be expected to delimit the spawning periods of individual fish in different ways. For example, the termination of the spawning period of a fish maturing early in the long spawning season of the species might be predominantly conditioned by its internal rhythm, as seems to be the case with *Notropis,* whereas that of a fish maturing late in the season could be the immediate result of the drop in temperature. The length of the over-all spawning season entails such a range in ages within the population that the internal rhythms of different fish would be out of phase much of the year unless synchronized by environmental factors within the spawning season, as well as by those of the non-breeding season, which appears to begin with the advent of subliminal low temperature.

It is inconceivable that the suppression of early phases of maturation by high temperature, alone or reinforced by long days, could be more than a retardation, for otherwise breeding would be prevented during most of the natural spawning season. The strong suppression of later phases of maturation by subliminal low temperature is understandable. A mere retardation of earlier phases of maturation by high temperature reinforced by long days, however, makes it possible to suppose that during the prolonged spawning season of *F. confluentus* the frequency or amplitude of oviposition, or both, as well as the numbers of spawners, may be greater toward the beginning and the end of the spawning season, when temperatures are lower and days are shorter. This combination of external factors has been shown to condition the spawning of salmonids in autumn (Hoover & Hubbard, 1937; *et alii*), but the influence of the same combination on the earliest spring spawners at high latitudes has not been studied experimentally. The above hypothesis could be tested statistically by sampling the gonosomatic indices of large numbers of female *F. confluentus* at intervals throughout the year. The problem is complicated, however, by (1) the probability that first spawners are recruited into the breeding population throughout the long spawning season, as they successively mature from the consequently staggered broods, some even displaced outside the spawning season through delayed hatching of stranded eggs, and by (2) the possibility that spawning may be partly tide-controlled (Harrington, 1959a), although tides are extremely variable in the Indian River region, where the present experimental material was obtained.

The field observations and experiments of Hubbs & Strawn (1957) on effects of daylength and temperature on the number of spawnings, number of eggs per spawning, and length of intervals between spawnings by the Greenthroat Darter, *Etheostoma lepidum,* provide the only other data cogent here concerning environmental influences on fish reproduction at low latitude.

Greenthroat Darters, living near springs at about 30° North Latitude where they were subject to an annual temperature range of 10° C. (14°-24° C.), when sampled throughout the year, were found in breeding condition on all dates of all but 1-2 months. Ripe fish were fewest in July, and young fish were lacking only in August. Downstream temperature extremes were greater (7°-35° C.), and where the annual range was 21.5° C., ripe fish were absent from May through October, presumably when temperatures exceeded 24° C. Experiments indicated an upper limit of egg production at 24°-27° C. These observations are suggestive with regard to the hypothesis that the reproduction activity of *F. confluentus* may slacken during the warmer, long-day, middle portion of its spawning season. Hubbs & Strawn found no evidence of a daylength influence on the amplitude or frequency of spawning. As they specify, however, the temperature was out of control and at or near the upper limit for egg production in their first series of experiments. Moreover, it is difficult to understand the rationale or to assess the results of subjecting the same fish to one daylength-temperature combination for 15 days and then to a different combination for 15 days. Since Greenthroat Darters spawn throughout all but the hottest, long-day months of the year, it might be supposed that the influence of daylength is at best subsidiary to that of temperature and possibly confined to reinforcing a retardation of ovogenesis by high-temperature extremes, as seems to be the case with *F. confluentus*. The demonstration of such a daylength effect may require an analysis of oocyte phases besides a stricter control of temperature, in view of the short annual range of temperatures to which these fish are habituated. Hubbs & Strawn made daily counts of eggs deposited on the sides of aquaria and on glass wool, but the count varied according to size of female, and egg eating was not controlled. Their second series of experiments was better controlled and of longer duration, and was evaluated in terms of variation in length of interspawning intervals, which these authors regarded as a more reliable criterion. Unfortunately there were few replications per daylength-temperature combination. Until these experiments are repeated with more refined techniques of control and evaluation, the results can be treated only as presumptive negative evidence of daylength influence on the spawning of Greenthroat Darters.

In *Fundulus confluentus,* the response of the gonosomatic index to maturation was mainly adterminal. It scarcely registered the advance of ovogenesis until the vanguard eggs were well into oocyte phase IV (*vide supra* and Text-fig. 3). It reflected maturation through the preceding oocyte phases only indirectly, as a time-measured end result. Earlier oocyte phases are important ecologically, however, and in different fishes are correlated with environmental factors in different ways. For instance, most high-latitude fishes winter over with ovaries in stage IV, but *Gasterosteus* does so with its ovaries in stage III (Kazanskii, 1951). Meien (1939) generalizes that at the end of the spawning season, the ovaries revert from stage VI to III in fishes with a long (interrupted) spawning season, but to stage II in fishes with a short (uninterrupted) one. This means that the post-spawning ovary of the former category is left with eggs in oocyte phases I-III, and that of the second with eggs in oocyte phases I-II only. Seasonal ovarian regression in the syrt', *Vimba vimba* L. var. *typicus,* for example, includes resorption of phase III oocytes (Sakun, 1957, figure 2), but in many other fishes, the eggs of this oocyte phase presumably are not resorbed at the end of the spawning season.

Although the above categories do not seem to accommodate *F. confluentus,* they attest to the ecological importance of earlier oocyte phases. In *F. confluentus,* ovarian maturation stage III is of peculiar interest because its vanguard eggs are in oocyte phase III, just before which the nucleoplasmic index begins to drop, within which the nucleus progressively diminishes in size and vitellogenesis begins, and at the end of which the nucleus stops shrinking and there is a change in the response of the oocytes to high temperature, *i.e.* they are no longer retarded in developing by sustained high temperature reinforced by long days but proceed rapidly to final maturity. The drop in the nucleoplasmic index and the reduction in nuclear diameter, which possibly involve emission into the cytoplasm of vitellogenic nuclear substances, leads inquiry from external environmental influences to levels of response among and within the cells of the target organ.

Summary and Conclusions

1. Specimens of Marsh Killifish, *Fundulus confluentus,* indigenous to 27° North Latitude, were subjected within the non-breeding season to four combinations of constant temperature (15 ± 1° C. or 30 ± 1° C.) and daylength (7 hours or 15 hours) for 45 days (December 15 to January 31).

2. The four experimental aquaria at the end

of the experiment contained 15, 17, 15 and 14 females, respectively, and 8-10 males each.

3. The effects of the variant treatments were measured in terms of the progress of the largest 50 eggs per fish through oocyte phases II to V. These four oocyte phases are defined by successive egg-diameter ranges. During phases II to IV, inclusive, the nuclear diameter was found to increase rapidly, gradually decrease, and then level off, respectively. As the eggs approach final maturity (phase V) the nucleus becomes obscured by yolk, and can no longer be measured in cleared eggs.

4. Oocyte phase II is the last phase of the primary (lesser) growth period, or the growth period 1 of authors. Oocyte phase III begins with the onset of vitellogenesis and is the first phase of the secondary (greater) growth period, or the growth period 2 of authors. It is the phase of primary accumulation of yolk, in the form of yolk vesicles (cytoplasmic vacuoles). Oocyte phase IV is the phase of accumulation of yolk in the form of lipid granules. Oocyte phase V comprises the stages of yolk consolidation and includes the definitive mature egg.

5. Each oocyte phase corresponds approximately with the phase of the most mature oocytes in ovaries belonging to the like-numbered ovarian maturation stage of Russian authors.

6. High temperature induced the later phases of maturation (oocyte phases IV through V), but retarded the earlier ones (II through III). Low temperature accelerated the earlier phases (II to mid-IV), but suppressed the later ones (mid-IV through V).

7. There was no incontrovertible evidence of daylength influence at low temperature or on later phases of maturation (oocyte phases IV through V) at high temperature, but the retardation of ovogenesis (oocyte phases II through III) by high temperature seemed to be strongly reinforced by long days.

8. The gonosomatic index scarcely reflected ovarian maturation until the largest 50 eggs were progressing from oocyte phase IV through V to final maturity.

9. The so-called refractory period, regarded as one of two alternate phases of an intrinsic reproductive rhythm in fishes, seems to parallel the passage of oocytes through the earlier oocyte phases, and nevertheless to be subject to external influence.

10. Because of the long breeding season (February into October) with its long-day, high-temperature middle portion, the retardation of early ovogenesis by high temperature reinforced by long days suggests that either or both the amplitude of oviposition and the number of spawners may be greater toward each end of the spawning period, when temperatures are lower and days are shorter. This hypothesis remains to be tested in the field.

11. The critical nature of oocyte phase III is indicated by the following: (a) the mean nuclear diameter shows consistently rapid increase prior to phase III, (b) the nucleoplasmic index drops abruptly just before phase III, (c) the mean nuclear diameter gradually decreases progressively throughout phase III, but levels off thereafter, (d) oocyte growth proceeds evenly and slowly up through phase III, accelerated by low temperature though unaffected by daylength at low temperature, but retarded by high temperature and still further retarded by long days in conjunction with high temperature, (e) maturation beyond phase III shows no evident responsiveness to daylength, advancing swiftly toward definitive maturity at high temperature, but not surpassing mid-phase IV at low temperature. These relationships acquire added significance from recent evidence favoring the hypothesis that in newt oocytes there is protein migration from cytoplasm to nucleus before vitellogenesis and in the opposite direction during vitellogenesis, and from the recent demonstration, in mouse cells, of nucleolar extrusion apparently related to the synthesis of material in the cytoplasm.

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